

Figure 1

Conservation of CCHC Type Retroviral Zinc Fingers (-Cys-(X)2-Cys-(X)4-His-(X)4-Cys-) Among Known Retroviruses

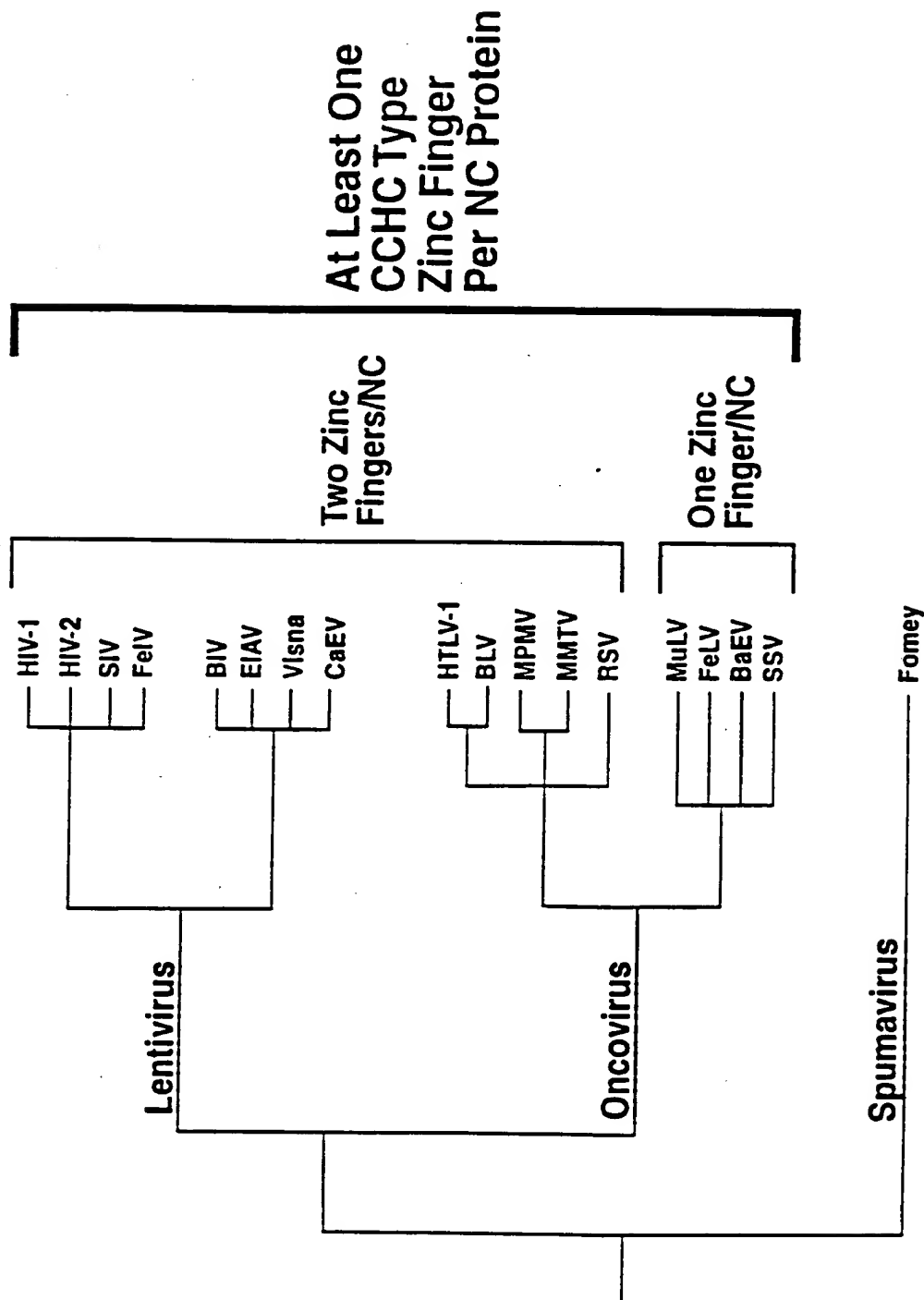


Figure 2

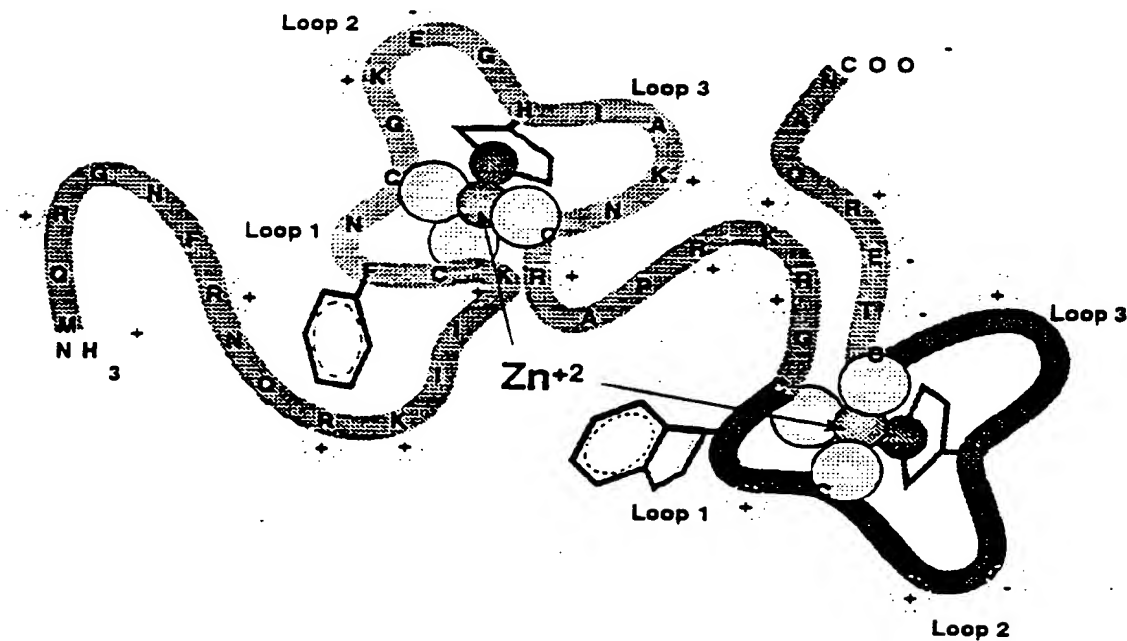
HIV-1_{MN} Nucleocapsid Protein



Total Residues.....55
 Basic Residues.....15
 Acid Residues..... 4
 Net Charge..... +11
 IEP.....10.77

Molecular
 Weight.....6451.5
 280nm Molar
 Absorption.6050

First Zinc Finger



Second Zinc Finger

Figure 3

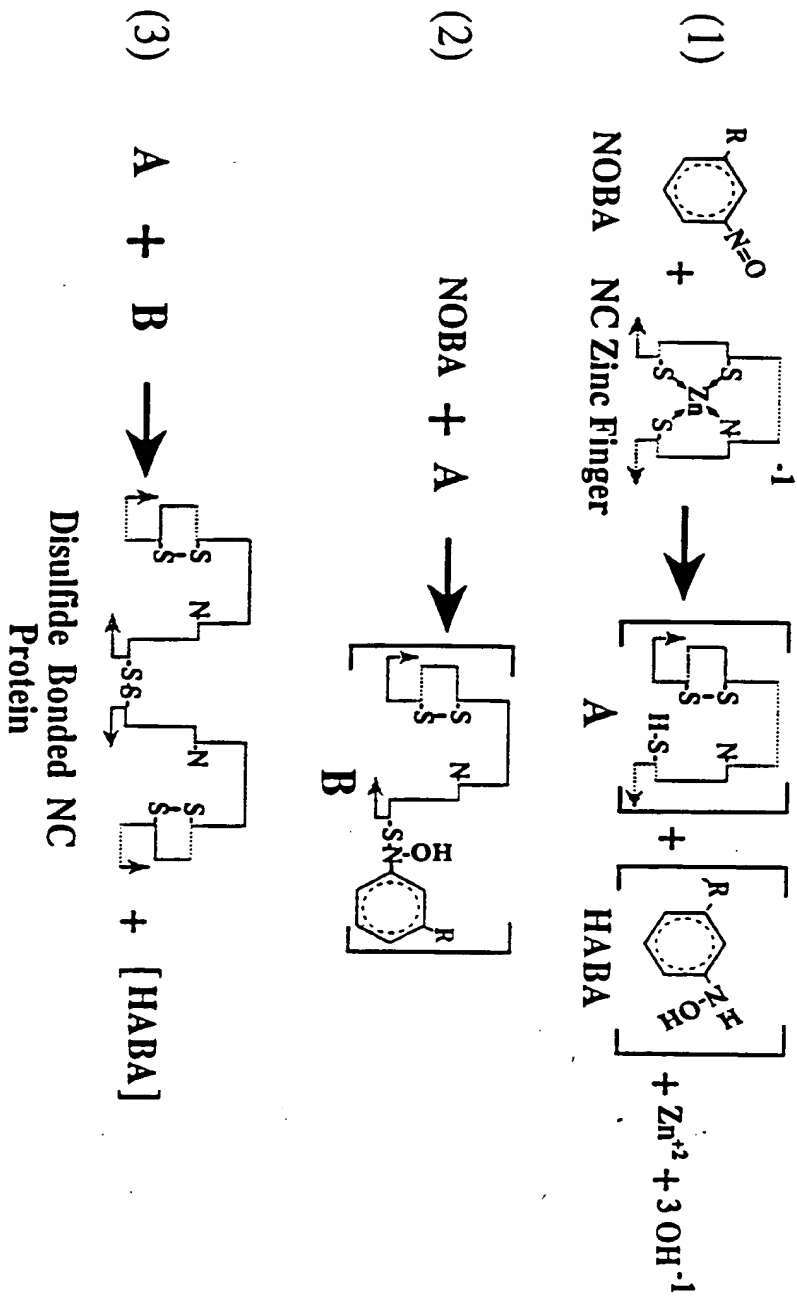
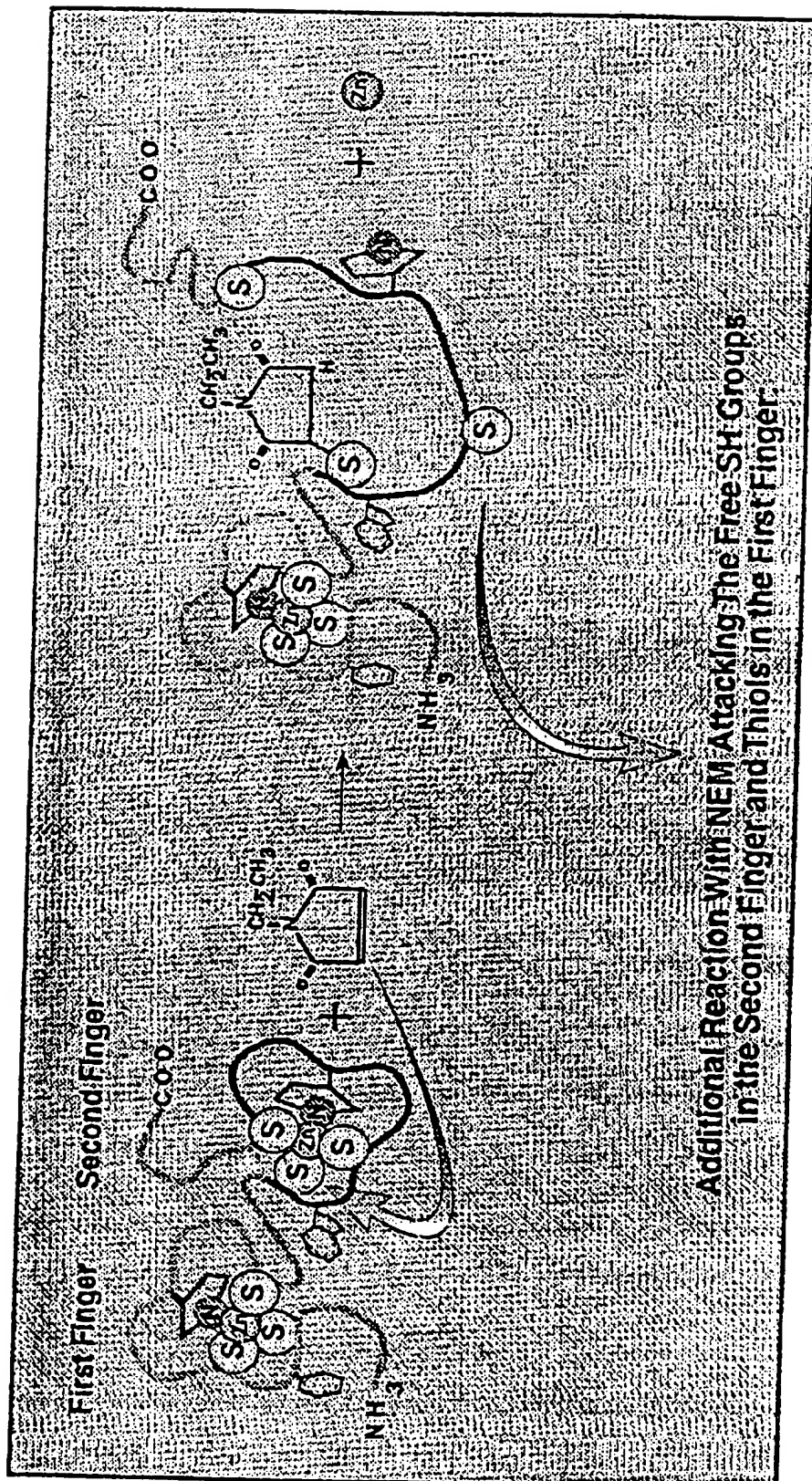


Figure 4

The Initial Reaction With NEM Modifies The First Cys Residue In The Second Finger

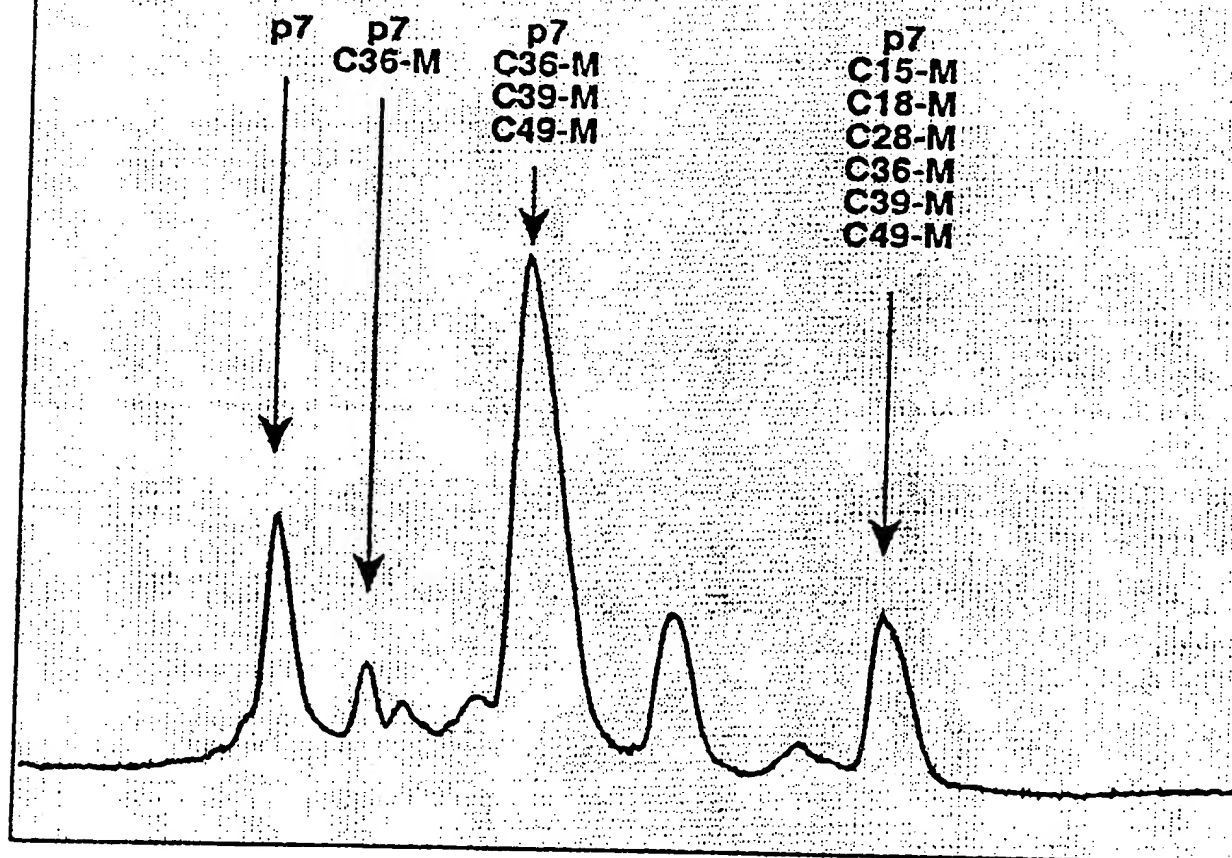


By reacting p7NC with limiting amounts of NEM and analyzing as in Fig. 6, it was determined that the first cysteine in the second zinc finger reacts fastest with the reagent. This is an example showing how the procedures have been used to investigate the reaction pathway and to determine the most reactive thiol in the NC protein.

FIGURE 5

Analysis of Reaction Products by HPLC

The positions of alkylated Cys residues were determined by sequence analysis of separated proteins and are indicated by the notation C#-M.



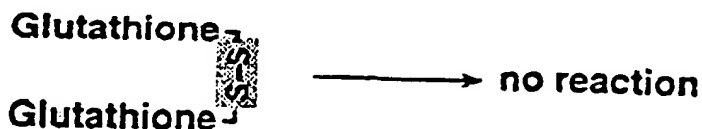
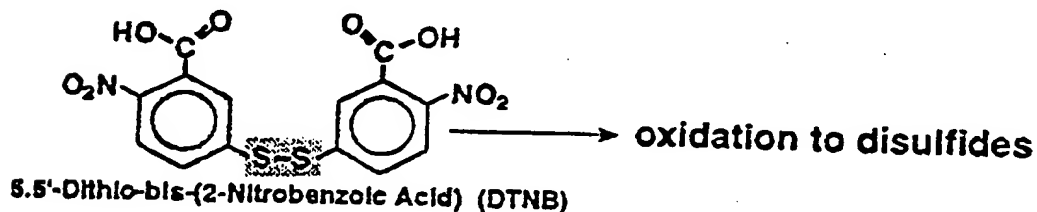
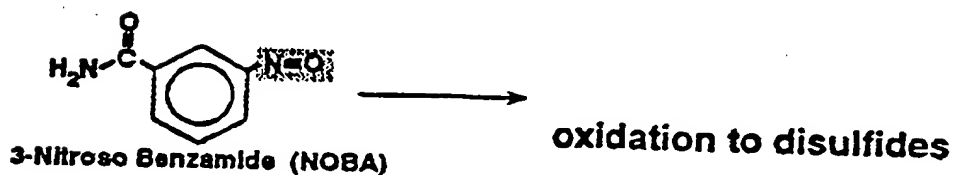
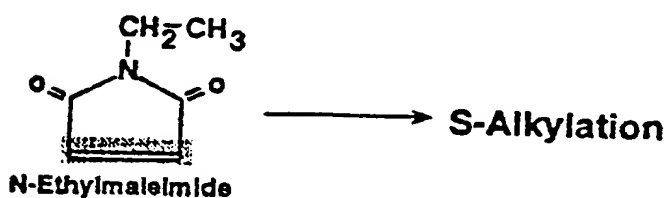
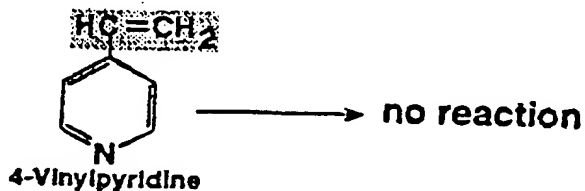
Reaction conditions: 62 mM p7NC + 744 mM NEM; pH 7.0, 60min. at RT.

Separation was accomplished by reversed phase HPLC using a C-18 μ -Bondapak (3.9 x 300 mm) column (Waters, Inc). Proteins were eluted at a flow rate of 1.0 ml/min. with gradients of acetonitrile (0-17, 20 min. 17-25, 120 min.) at pH 2.0 (0.05% trifluoroacetic acid). Proteins were detected by UV absorption at 206 nm.

FIGURE 6

REACTIONS OF HIV-1 NC RETROVIRAL CCHC ZINC FINGERS

Reagent Reaction With p7NC

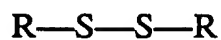


The reactive functional groups are shaded

Figure 7

Functional Groups Which React With Retroviral Zinc Fingers

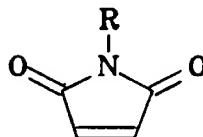
disulfides



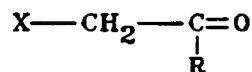
nitroso compounds



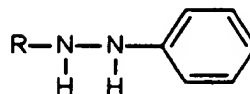
maleimides



α -halogenated ketones



phenylhydrazids



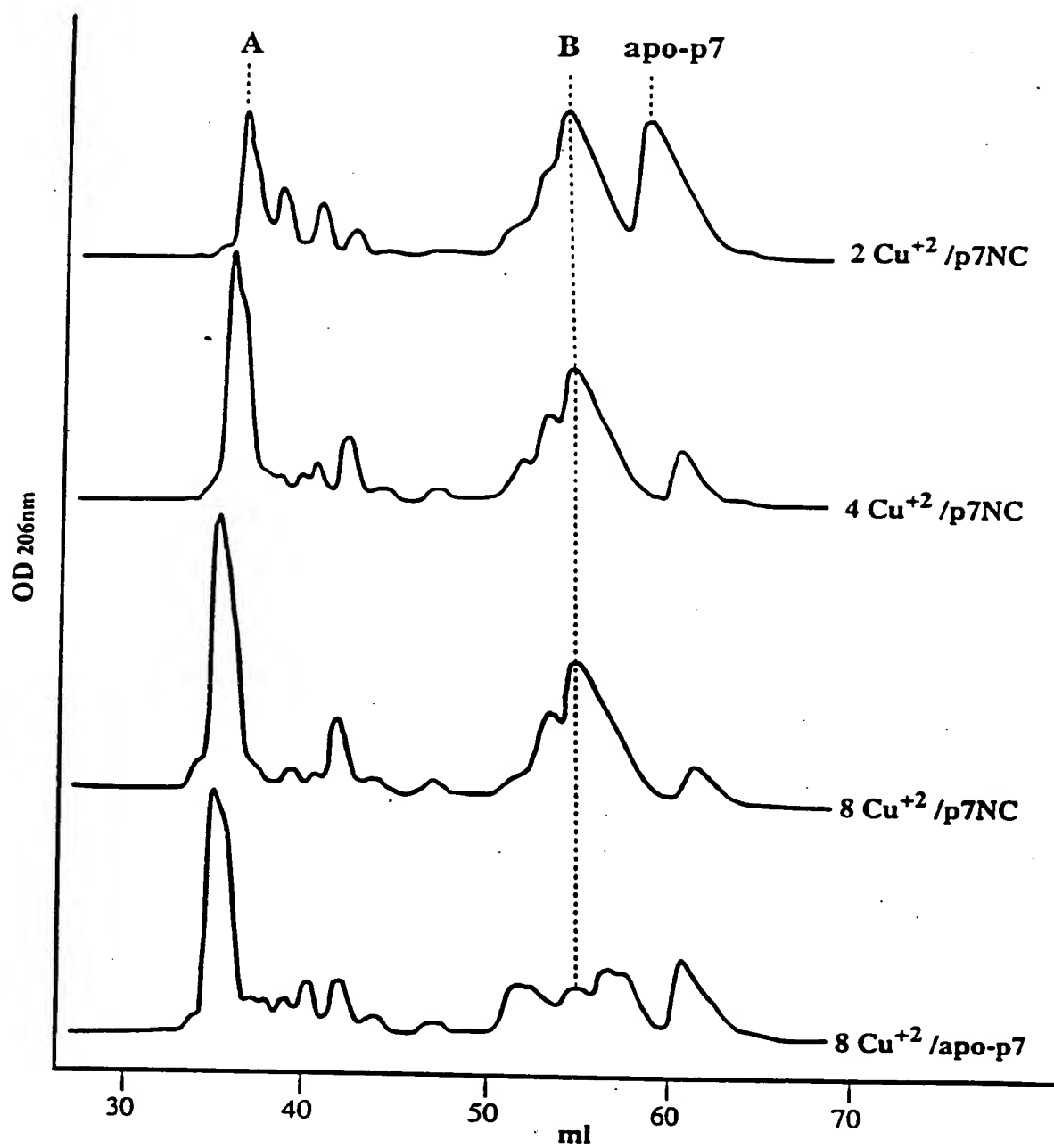
Nitric Oxide and Derivatives NO

cupric ions and complexes Cu^{+2}

ferric ions and complexes Fe^{+3}

wherein R is any atom or molecule, and X is selected from the group consisting of F, I, Br and Cl.

FIGURE 8



OD 206 nm

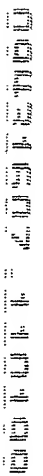
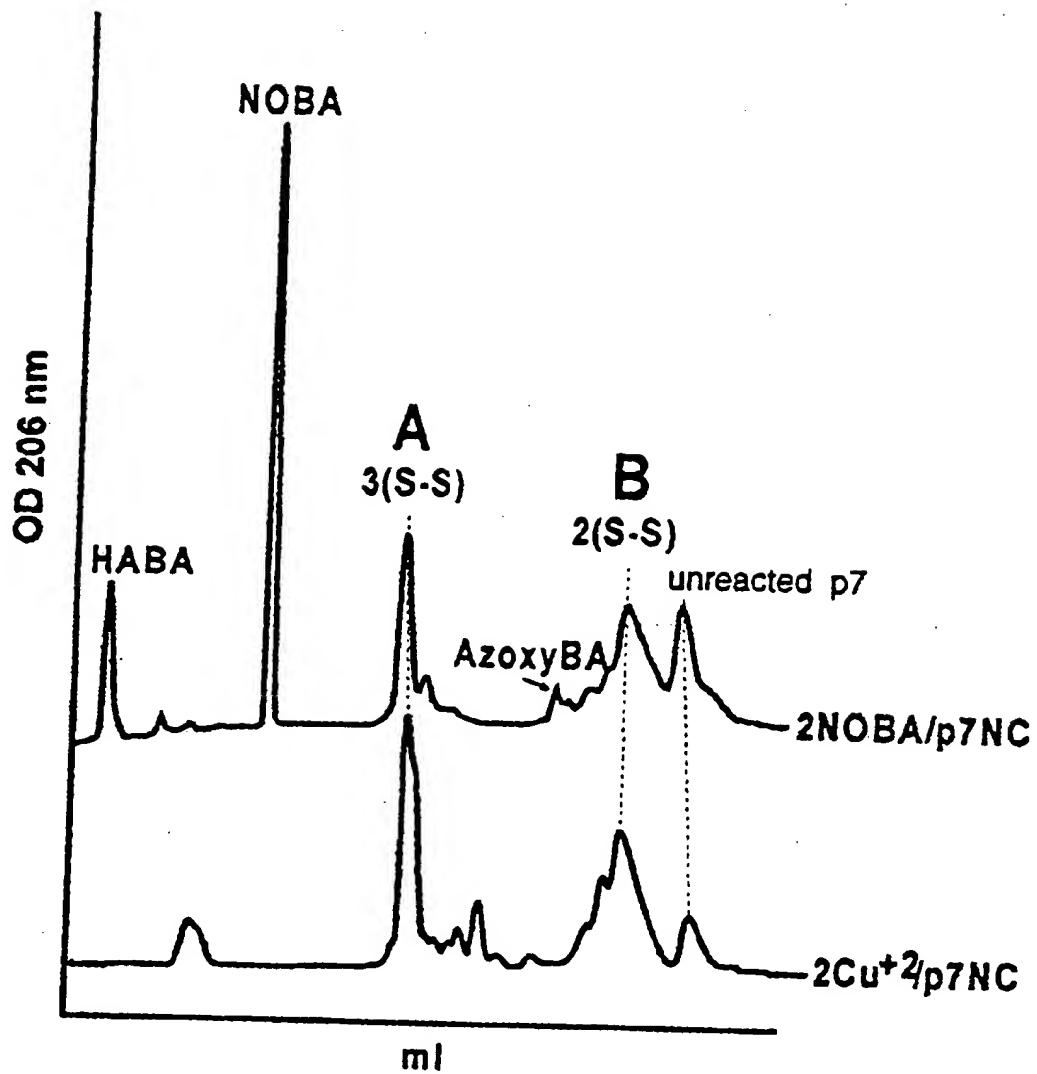


FIGURE 10



HPLC Chromatograms of NOBA and Cupric
Oxidation Products of p7NC

FIGURE 11

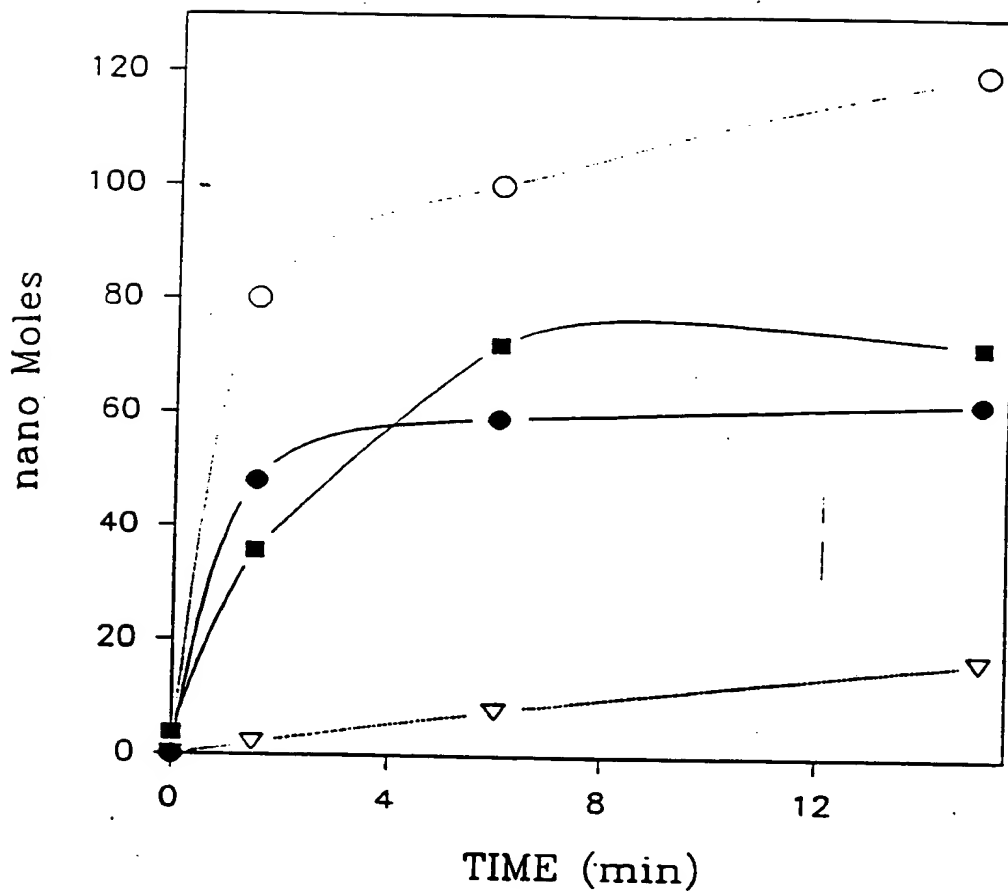
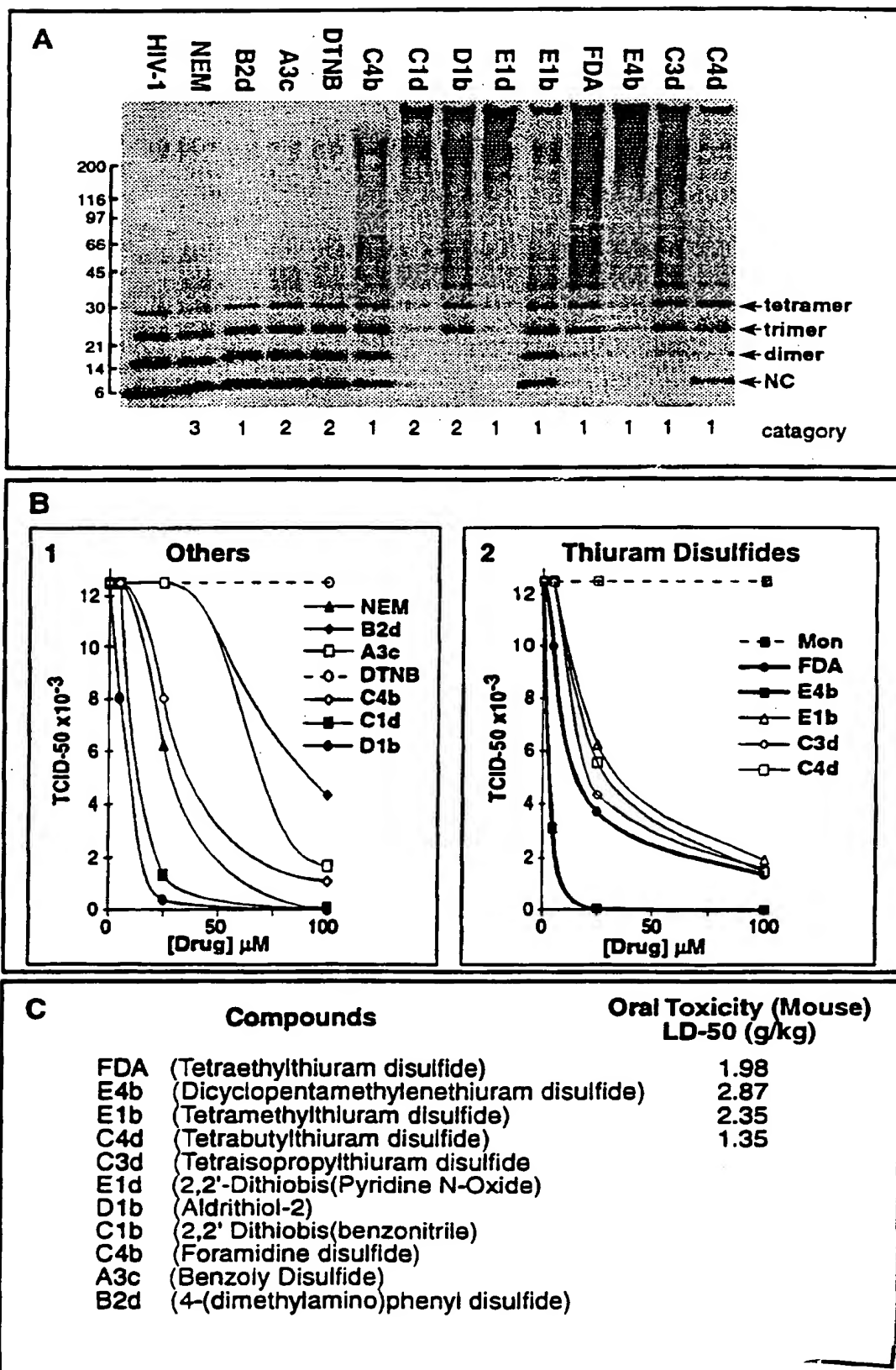




FIGURE 15

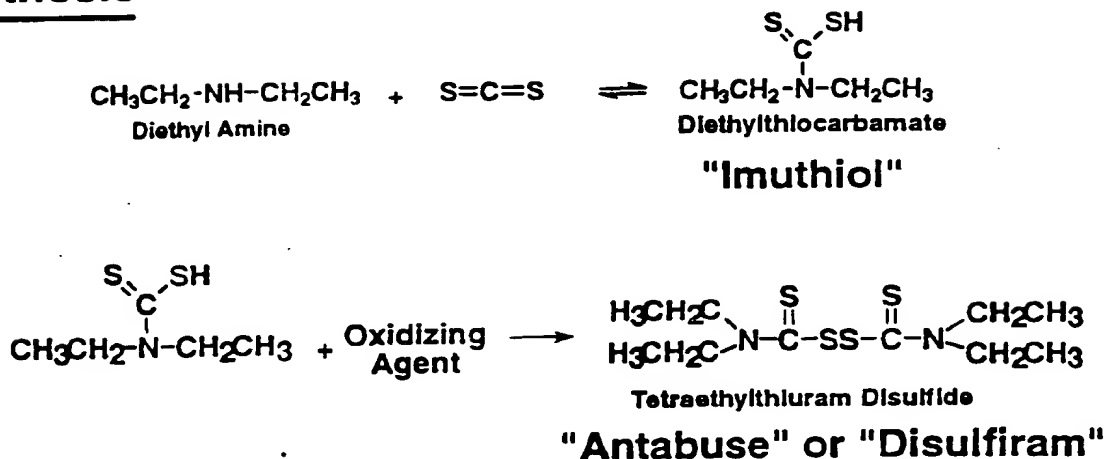


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FIGURE 14

Medical Use and Chemistry of Thiurams

Synthesis



General Reactions

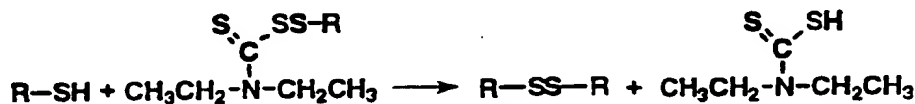
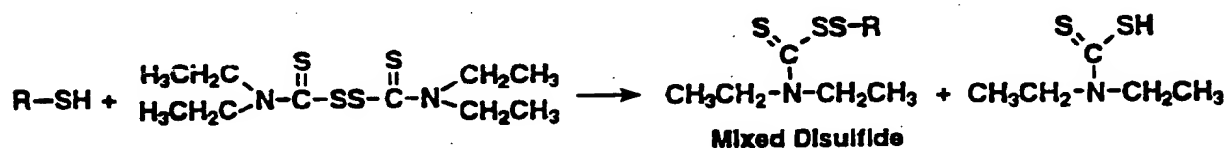
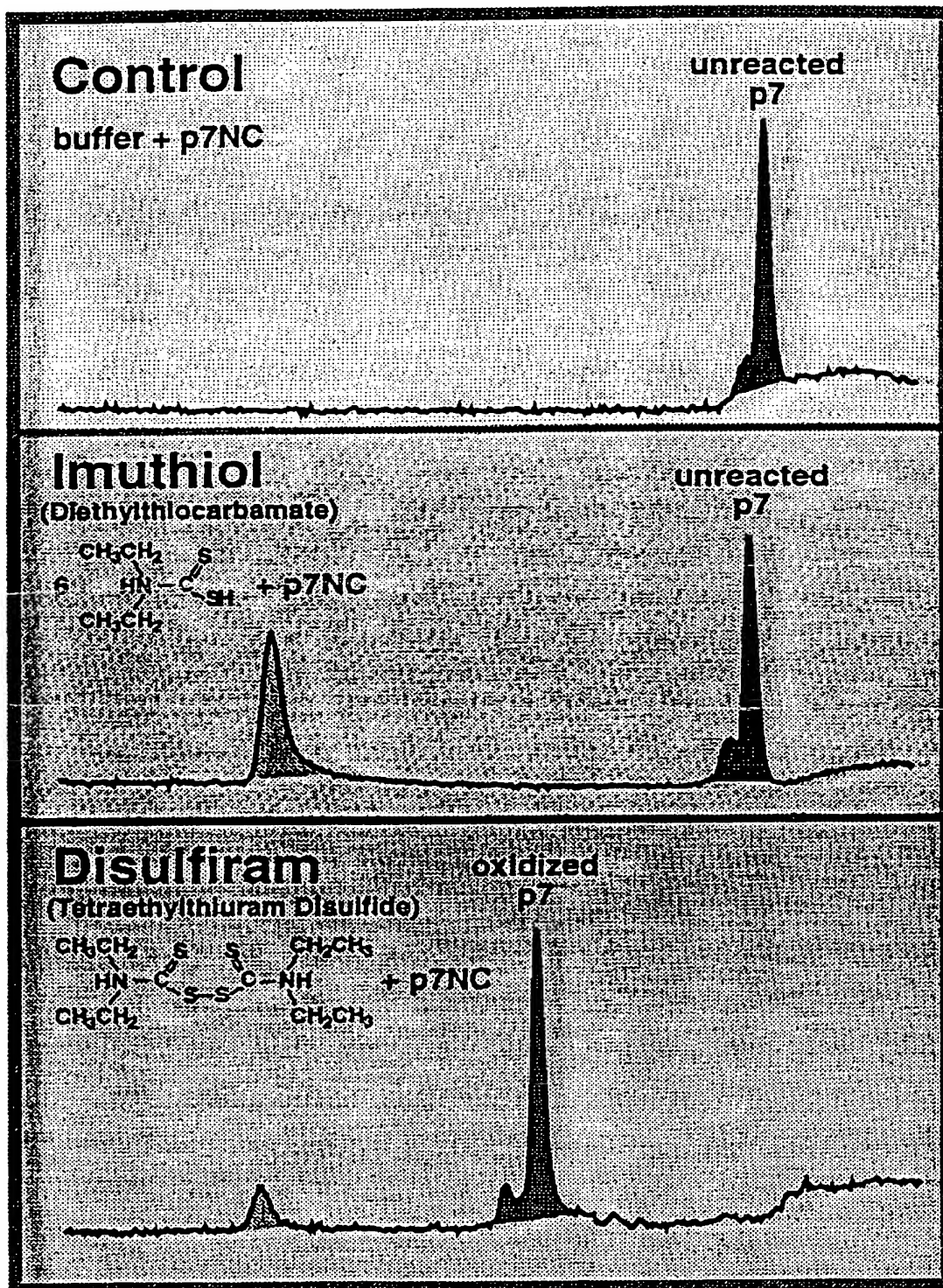


FIGURE 13

HPLC Analysis Of p7NC Reactions With Imuthiol and Disulfiram



00101-00100